International Journal of Surgery 10 (2012) 484–488

Contents lists available at SciVerse ScienceDirect

International Journal of Surgery

journal homepage: www.theijs.com

Original research

Thymoquinone ameliorates bacterial translocation and inflammatory response in rats with intestinal obstruction

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ARTICLE INFO

Article history:
Received 30 December 2011
Received in revised form
22 April 2012
Accepted 21 June 2012
Available online 28 June 2012

Keywords:
Thymoquinone
Intestinal obstruction
Bacterial translocation
Inflammatory response

ABSTRACT

Background: Intestinal obstructions might cause mucosal disruption, motility dysfunction, increasing intestinal volume, and intestinal bacterial overgrowth; it might also result in bacterial translocation. Thymoquinone is a bioactive substance that might affect antioxidant, anticancer, antimicrobial, anti-inflammatory, and immunomodulatory activities. In this study, we aimed to investigate the effectiveness of thymoquinone against bacterial translocation and inflammatory response induced by mechanical intestinal obstruction.

Methods: Thirty Wistar albino rats (200–250 g) were divided into three groups, as follows: Group 1 (sham), with only ileocaecal junction dissection; Group 2 (intestinal obstruction), with complete ileal ligation; Group 3 (intestinal obstruction + thymoquinone), with complete ileal ligation and given 10 mg/kg thymoquinone intraperitoneally. After 24 h, the rats were sacrificed by taking blood from the heart for biochemical analyses. Peritoneal swab cultures and the liver, mesenteric lymph nodes, spleen, and ileum were collected for microbiological and histopathological examinations.

Results: Thymoquinone reduced the secretion of inflammatory cytokines, oxidative damage, and bacterial translocation, and prevented inflammatory changes in intestine and liver; it also significantly ameliorated intestinal mucosal damage after intestinal obstruction ($P < 0.05$).

Conclusions: Thymoquinone was found effective in successfully controlling bacterial translocation and improving intestinal barrier function.

1. Introduction

The gut has an important role as a regulatory organ in sepsis and multiorgan dysfunction syndrome (MODS). Bacterial translocation from the guts might occur under several conditions, such as intestinal obstruction (IO), intestinal ischemia, obstructive jaundice, burns, and hemorrhagic shock.[1–3] IO is a common lethal abdominal emergency that might cause a high mortality rate, often depending on MODS. The major factors that contribute to the development of MODS in IO are bacterial translocation (BT) with septic peritonitis.[4,5] IO leads to mucosal disruption, motility dysfunction, increasing intestinal volume, and intestinal bacterial overgrowth; it might also result in BT.[6]

Nigella sativa L. (family Ranunculaceae) is also known as black seed or black cumin. Thymoquinone (TQ; 2-isopropyl-5-methyl-1,4-benzoquinone) is the bioactive component of *N. sativa* L. that might be involved in antioxidant, anticancer, antimicrobial, anti-inflammatory, and immunomodulatory activities.[7–9]

It has been reported that the oil and other TQ-active components of *N. sativa* L. seeds have antiviral, antifungal, and anti-helminthic antimicrobial effects.[8,10,11] Some of these antimicrobial effects are thought to be associated with TQ's immunomodulatory effects.[8,12]

In this study, we aimed to investigate the effectiveness of TQ in microbiological, biochemical, and histopathological studies on reducing the severity of inflammatory response and bacterial translocation induced by mechanical IO.

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1743-9191/$ – see front matter
http://dx.doi.org/10.1016/j.ijsu.2012.06.006

2. Materials and methods

2.1. Chemical

TQ was purchased from Sigma Chemical Co. (London, UK, Catalog no.: 274666; CAS No.: 490-91-5) and dissolved in dimethyl sulfoxide.

2.2. Animals

Our study included 30 Wistar albino rats, each weighing 200–250 g, that were obtained from the Dicle University Health Sciences Application and Research Center. All experimental procedures complied with its Guide for the Care and Use of Laboratory Animals. Rats were housed under standard conditions in an air-conditioned room with 12-h light and dark cycles, with constant temperature (22 ± 2 °C). The rats were housed in cages, and allowed free access to standard rat chow and water before the experiments. The animals were fasted overnight the day before surgery, but had access to water ad libitum.

Thirty Wistar albino rats were divided into the following three groups ($n = 10$). [1] Group 1 (Sham): only ileal manipulation was performed and no drug was given. [2] Group 2 (IO): ileal manipulation and ligation, 1 cm proximal to the caecum with 3–0 silk suture were performed, and no drug was given. [3] Group 3 (IO + TQ): ileal manipulation and ligation, 1 cm proximal to the caecum with 3-0 silk suture, were performed, and TQ was given at a dose of 10 mg/kg via the intraperitoneal route at the end of the experimental study.[12]

2.3. Experimental protocol

Rats were anesthetized with 50 mg/kg ketamine hydrochloride (Ketalar®; Parke Davis, Eczacibasi, Istanbul, Turkey) and 10 mg kg xylazine (Rompun®; Bayer AG, Leverkusen, Germany) via intramuscular injection, and the experimental procedure was initiated. A 10% povidone-iodine solution was used for shaved skin cleansing. A midline incision was performed. The terminal ileum was exposed. The distal ileum was ligated with 3-0 silk suture at 1 cm proximal to the caecum, obstructing the passage but not inhibiting the circulation of the vessels. Saline (2 ml) was given via the intraperitoneal route, and the incision was closed in a single layer.

After a period of 24 h, the rats were again anesthetized and sacrificed by taking blood from the heart for biochemical analyses. Immediately, a thoracoabdominal midline incision was performed under completely sterile conditions. Before taking blood and tissue samples, a peritoneal swab was taken for culture from the peritoneal cavity with a sterile swab stick. For microbiological analyses, 1 ml blood samples from the inferior vena cava, mesenteric lymph nodes (MLNs), liver, and spleen tissues were collected. A 3 cm diameter of ileal segment proximal to the ligation was removed for histopathological evaluation. MLNs and liver tissue were obtained for histopathological examinations. Serum samples were obtained from the centrifuged blood samples and rapidly transferred to plastic ependorf covered tubes for biochemical analyses and stored at −80 °C in a deep freezer. In addition, the tissues were taken for histopathological evaluation; foreign tissue residues and blood were removed, washed with saline, and put into plastic containers holding 10% formaldehyde solution.

2.4. Microbiological evaluation

Blood samples were obtained from the inferior vena cava and cultured aerobically and anaerobically using BacTec™ Peds bottles (Becton–Dickinson Diagnostic Inc., Sparks, MD, USA). Identification was done with the BD-Phoenix 100 TM system. Peritoneal swabs and positive cultures were plated out on blood agar, eosin methylene blue agar, chocolate agar, or Sabouraud dextrose agar. At the same time, MLNs, spleen, and liver were removed and placed in sterile glass bottles containing sterile brain–heart infusion medium. The bottles were reweighed and tissue homogenates were prepared in 2 ml brain–heart infusion medium using a sterile mortar and pestle. A portion (0.1 ml) of each homogenate was cultured on blood agar, eosin methylene blue agar, chocolate agar, or Sabouraud-dextrose agar. All agar plates were examined after 24 h and after 48 h of incubation at 37 °C. The incidence of bacterial translocation was calculated by determining the number of rats with positive bacterial culture divided by the total number of rats studied.

2.5. Biochemical analyses

Total oxidant activity (TOA), total antioxidant capacity (TAC), paraxonase (PONX), tumor necrosis factor-alpha (TNFα), interleukin-6 (IL6), interleukin-1 beta (IL1β), and C-reactive protein (CRP) analyses were performed on the blood samples.

2.5.1. Measurement of TOA

The TOA of supernatant fractions was determined using a novel automated measurement method, developed by Erel.[13] Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to produce ferric ions. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ions produce a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of nmol H_2O_2 Equiv./mg protein.

2.5.2. Measurement of TAC

The TAC of supernatant fractions was determined using a novel automated measurement method developed by Erel.[14] In this method, the hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution present in reagent 1 is mixed with hydrogen peroxide in reagent 2. The sequential radicals produced, such as the brown brown-colored dianisidinyl radical cation, are also potent radicals. Using this method, the antioxidative effect of the sample against the potent free radical reactions, which are initiated by the hydroxyl radical produced, is measured. The assay has excellent precision values, lower than 3%. The results are expressed as nmol Trolox Equiv./mg protein.

2.5.3. Measurement of the PONX

Serum PONX levels were measured spectrophotometrically by modified Eckerson method.[15] Initial rates of hydrolysis of paraoxon (0.0-diethyl-0-p-nitrophenylphosphate; Sigma Chemical Co.; London, UK) were determined by measuring liberated p-nitrophenol at 405 nm at 37 °C. The results were expressed as U/L.[14]

2.5.4. Measurement of TNFα, IL6, IL1β, and CRP

TNFα, IL6 and IL1β (Diasource, Nivelles, Belgium) were determined using the enzyme amplified sensitivity immunoassay method. The serum Hs-CRP levels (DRG, NJ, USA) were determined using the enzyme-linked immunosorbent assay method.

2.6. Histopathological assessment

All blinded histopathological evaluations were performed by the same pathologist. Ileal segment, MLNs, and liver tissues were put into the 10% formalin solution in paraffin blocks from which 4 μm sections were sliced. Tissues were stained with hematoxylin-eosin, and standard protocols were applied. Ileal segments, MLNs, and liver samples were examined for inflammatory cell infiltrate grading, and ileal segments were examined for ileal mucosal injury score by an expert pathologist using light microscopy (Nikon ECLIPSE 80i). In concordance with the literature, the changes were graded as follows; Grade 0, no changes; Grade 1, mild changes; Grade 2, moderate changes; Grade 3, severe changes.[4,16] In addition, all tissue samples were examined under light microscopy by staining Giemsa for evaluation of bacterial translocation.

2.7. Statistical analysis

Statistical analysis was performed by SPSS for Windows 11.5 (SPSS Inc., Chicago, IL, USA). Data were presented as mean (minimum, maximum) values for biochemical values. Groups were compared by using the nonparametric Kruskal–Wallis test. The Mann–Whitney *U* test was used for binary comparisons of continuous variables, and the Chi-square test was used for categorical variables. A *P*-value of less than 0.05 was considered significant.

3. Results

All animals survived throughout the experimental procedures. Biochemical results are summarized in Table 1. IO was significantly associated with oxidative stress. Serum PONX and TOA levels were different in each group, but TAC levels were similar in all groups. PONX activity was lower in the IO group than either the S group or IO + TQ group. TOA levels increased in the IO group, but treatment with TQ significantly prevented the increase of TOA levels after IO.

Table 1
Biochemical results of the groups.

Groups	S ($n = 10$)	IO ($n = 10$)	IO + TQ ($n = 10$)
PONX (U/L)	35.54 ± 8.52	18.68 ± 4.26[a]	47.94 ± 21.37[b]
TAS (mmol Trolox Eq./L)	0.72 ± 0.059	0.71 ± 0.09	0.88 ± 0.21
TOS (μmol H_2O_2 Equiv./L)	12.14 ± 1.21	33.52 ± 10.58[a]	16.20 ± 3.45[a,b]
TNF-α (pg/mL)	1.93 ± 0.86	7.59 ± 1.72[a]	1.65 ± 0.98[b]
IL-6 (pg/mL)	31.25 ± 8.45	65.83 ± 20.44[a]	36.45 ± 15.50[c]
IL-1β (pg/mL)	0.47 ± 0.11	1.62 ± 0.59[a]	0.91 ± 0.87[c]
CRP (mg/L)	30.46 ± 4.64	165.27 ± 41.06[a]	68.15 ± 13.56[a,b]

Data were given as Mean ± SD.
[a] Significantly different when compared with S group, ($p \leq 0.001$).
[b] Significantly different when compared with IO group ($p \leq 0.001$).
[c] Significantly different when compared with IO group ($p = 0.01$).

Table 2
Histopathological grading of the groups.

Groups	S ($n = 10$)	IO ($n = 10$)	IO + TQ ($n = 10$)
Liver inflammation score	0.11 ± 0.31	1.34 ± 0.47[a]	0.60 ± 0.50[b,d]
MLN size (mm)	0.25 ± 0.08	0.19 ± 0.078[b]	0.30 ± 0.14[e]
MLN inflammation score	1.50 ± 0.53	2.50 ± 0.53[c]	1.60 ± 0.42[d]
Ileum inflammation score	1.00 ± 0.00	2.59 ± 0.51[a]	1.20 ± 0.42[f]
Ileal mucosal damage score	0.00 ± 0.00	2.29 ± 0.69[a]	1.20 ± 0.42[d]

Data were given as Mean ± SD.
[a] Significantly different when compared with S group, ($p < 0.001$).
[b] Significantly different when compared with S group, ($p < 0.05$).
[c] Significantly different when compared with S group, ($p < 0.01$).
[d] Significantly different when compared with IO group ($p < 0.01$).
[e] Significantly different when compared with IO group ($p < 0.05$).
[f] Significantly different when compared with IO group ($p < 0.001$).

In IO + TQ group, the inflammatory cytokines TNF-α, IL-6, IL-1β, and CRP increased after IO. Treatment with TQ significantly decreased all these cytokines, compared with the IO group.

The histopathological gradings of the liver, MLNs, and ileum are summarized in Table 2. The inflammation scores of the ileum ($P < 0.001$), liver ($P < 0.001$), and MLN ($P = 0.03$) were higher in the IO group than in the S group. The ileal mucosal damage score was also significantly higher in the IO group than in the S group ($P < 0.001$). The MLN diameter was significantly lower in the IO group than in the S group ($P = 0.035$) and the IO + TQ group ($P = 0.043$). The inflammation scores of the ileum ($P < 0.001$), liver ($P = 0.007$), and MLN ($P = 0.007$) were lower in the IO + TQ group than in the IO group. Shorter lengths of intestinal villus height were found in IO group. However, treatment with TQ prevented intestinal villus height shortening and had protective effects on the intestinal mucosal damage scores ($P = 0.003$; Figs. 1–3). With the examination of ileal tissue samples under a light microscope by staining Giemsa, we were able to determine bacterias infiltration to the epithelium of the intestinal mucosa in only 3 rats of IO group (Fig. 4).

The culture results are summarized in Table 3 as the number of rats with positive bacterial culture divided by the total number of rats. There was no difference between the groups in terms of peritoneal cultures. The blood ($P = 0.002$), liver ($P < 0.001$), spleen ($P = 0.007$), and MLN ($P = 0.007$) cultures were significantly positive in the IO group, compared with the S group. The positive cultures of the blood, liver, spleen, and MLN cultures were significantly higher in the IO group than in the IO + TQ group, depending on the treatment with TQ ($P = 0.023$).

4. Discussion

The mortality rate in IO cases is being reduced by the ongoing development of treatment methods. However, there are no established medical (pharmacological) treatment methods that are known to be useful, except antibiotics. The main factor affecting the course of disease in IO is fluid loss. One of the main causes of fluid loss is inflammatory changes occurring in the proximal part of the obstructed bowel segment.[17] At one point, the integrity of intestinal mucosa is disturbed after IO. Intestinal mucosa loses its barrier function against bacteria and endotoxins, which leads to BT and systemic infections.[18,19] Controlling only intestinal flora is not enough to prevent the development of BT, when the intestinal mucosal barrier function is eliminated as a result of IO.[4] IO is a suitable model for obtaining a high proportion of BT in liver, MLNs, and blood circulation at an early phase after the insult.[3] In this study, we measured the effects of TQ on BT in rats with IO.

Stasis caused by IO is known to facilitate BT with a variety of mechanisms. Hemodynamic changes, hypotension, and vasoactive agents that affect intestinal perfusion might induce the development of BT.[4,18] Enochsson et al.[19] reported that regional blood flow in both obstructed and nonobstructed small bowels had been significantly reduced by an intra-intestinal pressure of 40 mmHg. A pressure of 20 mmHg damaged the circulation in the obstructed small bowel, while no similar changes were seen in the non-obstructed group. In addition, the ischemic injury of intestinal mucosa leads to the development of BT. Mesenteric ischemia damages intestinal endothelial/epithelial integrity, and causes the loss of intestinal mucosal barrier function.[3,20] The increase in bacterial translocation after IO might be related to ileal mucosal damage, and might also result in the reduction of villus height. In our study, we have determined that there was a significant decrease in BT and an important increase in the mean villus height after TQ treatment.

Enlargement of MLNs is often related to primary mesenteric adenitis, frequently caused by viral infections, and may also be secondary to a detectable or known intra-abdominal inflammatory processes.[21] In sharp contrast, despite of the MLNs inflammation were more intensive in IO group, the diameter of MLNs in IO group was smaller than the other groups in our study. These results may have been related to the method of tissue sampling.

The most common isolated bacteria from systemic circulation and the tissues with BT is *Escherichia coli*. In some studies, the antibacterial activity of TQ against to several bacterial strains was reported, including *E. coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Peudomonas aeruginosa*, and *Bacillus subtilis*, as well as against *Candida albicans* and fungus.[8,10,22,23] *E. coli* and *Proteus* spp. were found to be the most commonly translocated bacteria in our study. The positive cultures in the MLN, spleen, and liver in the treatment group were significantly lower than those in the IO group. These results support the conclusion that TQ is effective inactivates gram-negative and gram-positive bacteria. TQ was found to be an effective antimicrobial agent against several of the microorganisms previously studied, but it remains unclear which mechanisms explain TQ's effectiveness. Further studies are needed to determine these mechanisms and open the way for the use of TQ in cotherapeutic treatments.

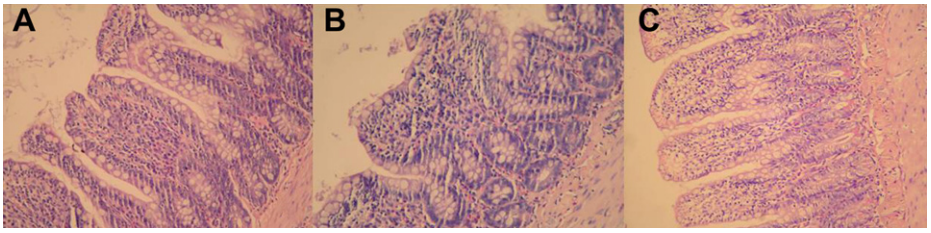

Fig. 1. Microscopic appearance of the effects of TQ on the ileal inflammation and mucosal injury after IO. A: Sham group. Minimal mucosal inflammation (H&E stain, ×200); B: IO group. Subtotal villous atrophy and epithelial degenerative changes in the intestinal mucosa with severe inflammation and edema (H&E stain, ×200); C: IO + TQ group. Slight blunting of the ends of the mucosal villi with mild to moderate inflammation is seen (H&E stain, ×200).

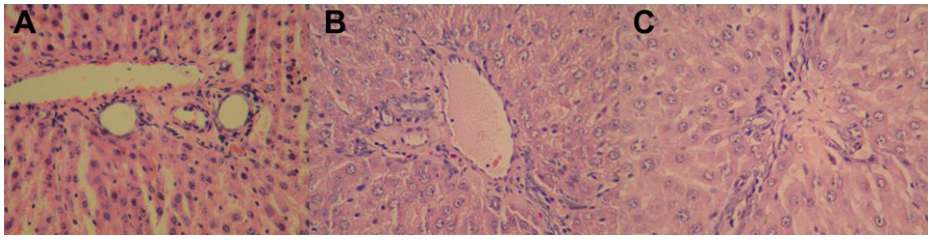

Fig. 2. The effects of TQ on the liver inflammation after IO. A: Sham group. Mild edema in the liver parenchima without inflammation (H&E stain, ×200); B: IO group. Moderate portal inflammation and edema in the liver (H&E stain, ×200); C: IO + TQ group. Mild portal inflammation and edema in the liver are seen (H&E stain, ×200).

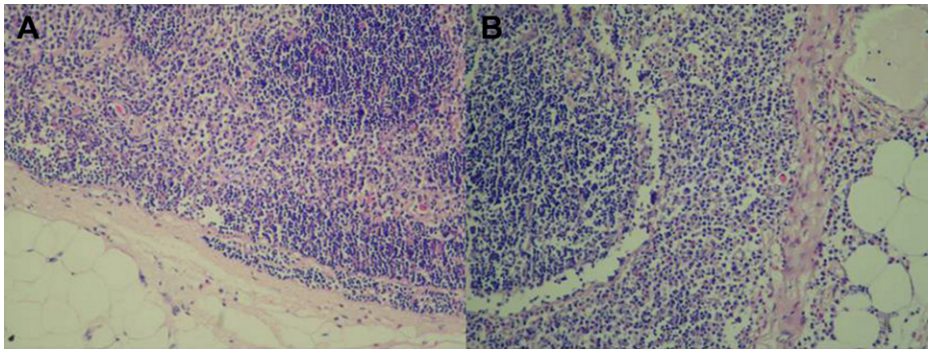

Fig. 3. The effects of IO on MLNs. A: Lymph node in normal architecture (H&E stain, ×200); B: Lymph node infiltrated with inflammatory cells including eosinophils (H&E stain, ×200).

In parallel with the previously determined relation between CRP and BT, Moore[24] has reported that the ischemic variant leads to the release of mediators that might cause systemic inflammatory response. CRP is a nonselective and reliable diagnostic indicator in suspected cases, and an important predictor of BT severity.[5] In this study, we showed that the CRP levels were significantly lower in the TQ treatment group than the IO group. These findings support the conclusion that TQ has anti-inflammatory and antibacterial effects on these changes cause by BT.

Reactive oxygen species, including nitric oxide and superoxide anions that emerge following IO, can lead to oxidative cellular damage. The protective effects of TQ in IO and sepsis are thought to be due to its function as an antioxidant in biological redox cycling between quinine and hydroquinone. The biological components might be protected from the harmful effects of reactive oxygen species by this antioxidant function.[25] The immunotherapeutic effects of TQ are attributed to its antihistaminic, antitoxic, and anti-inflammatory functions, and these might explain the efficacy of TQ as an antimicrobial and anticancer agent. TQ can alter the trafficking of the inflammatory cells by modulating the expression of chemokines and/or adhesion molecules, and it has inhibitory effects on the inflammatory immune response.[8] Sayed et al.[26] have reported that TQ exhibits its antioxidant activity with nuclear factor kappa B inhibition, which is a main transcription factor for the production of many inflammatory cytokines.[27] The inhibition of the transcription of many inflammatory cytokines, such as IL-1β and IL-8, and the enhancement of chemokines by TQ might explain its anti-inflammatory effects.[8,28] In addition, IL-6 induces inflammatory responses and plays a crucial role in acute phase reactions.[29] Like IL-6, TNF-α plays a critical role in the initiation and continuation of acute and chronic intestinal inflammation, and in mucosal inflammation as a focal point of the inflammatory cascade.[30] In our study, we demonstrated that the serum levels of cytokines such as TNF-α, IL-6, and IL-1β, which increased the inflammation and led to cellular damage, were significantly lower in the TQ treatment group than in

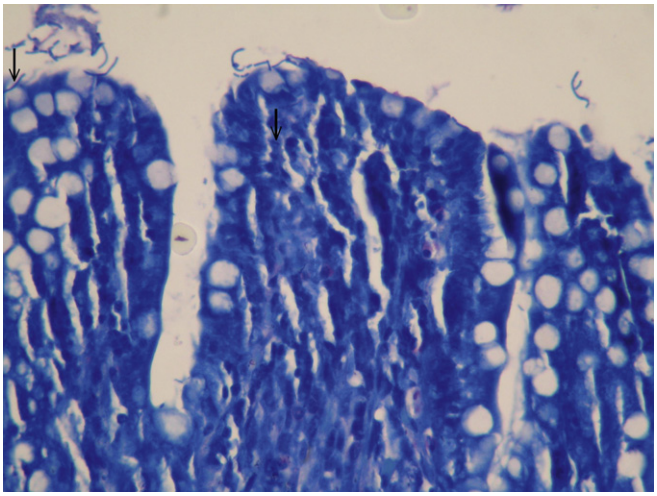

Fig. 4. Intestinal mucosa showing subtotal villous atrophy with bacteria infiltrated in the epithelium (Giemsa stain, ×400).

Table 3
Microbiological culture results of groups.

Groups	S ($n = 10$)	IO ($n = 10$)	IO + TQ ($n = 10$)
Blood culture (c/d)	0/10	8/10[a]	2/10[c]
Liver culture (d/e)	0/10	9/10[b]	3/10[c]
Spleen culture (d/e)	1/10	8/10[a]	2/10[c]
MLN culture (d/e)	2/10	9/10[a]	3/10[c]
Peritoneal culture (d/e)	2/10	4/10	2/10

Data were given as (d/e) = positive culture/total rat of group.
[a] Significantly different when compared with S group ($p < 0.01$).
[b] Significantly different when compared with S group ($p < 0.001$).
[c] Significantly different when compared with IO group ($p < 0.05$).

the IO group. These results might explain and support claims for TQ's anti-inflammatory activity and protection against BT.

5. Conclusion

TQ successfully controlled BT and improved intestinal barrier function. Our study demonstrated that TQ might have potential positive effects on clinical problems due to IO. These studies could determine the potential medical use of TQ, in combination with selected antimicrobial drugs, against bacterial infection. We have demonstrated the potential protective effects of TQ as an antioxidant, antimicrobial, and anti-inflammatory agent during the development of BT in an experimental IO rat model; however, it remains unclear which mechanism will ultimately be found the most effective against BT and inflammatory responses, one of the limitations of our study. Also, the small number of animal in each group is the other limitation in our study. Therefore, further studies are needed to explain the mechanisms by which TQ prevents BT.

Ethical approval

The experimental manipulations and surgical operations in this study were approved by the Committee of Experimental Animals of Dicle University.

Sources of funding

None.

Conflicts of interest

None.

Author contribution

Murat Kapan: Design, Writing, Literature Search, Collection, submission process.

Recep Tekin: Microbiological analyses, Writing, Litrature search/collection.

Akın Onder: Writing, Design, Referencing style.

Ugur Firat: Histopathological analyses, Writing,

Osman Evliyaoglu: Biochemical analyses, Writing,

Fatih Taskesen and Zulfu Arikanoglu: comments/corrections and final approval before submission for publications.

Acknowledgment

The authors would like to thank Ms. Gulsen Yilmaz, MD for her kind advice and support.

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